communication. Claim 32 has been cancelled without prejudice, and Claim 42 substituted therefore in order to more clearly define the metes and bounds of the invention.

Claims 23, 27, 38, and 39 have been amended to more clearly define the metes and bounds of the claimed invention. Claims 25 and 26 have been amended to recite specific pyruvate kinase genes. Basis for these amendments to Claims 25 and 26 can be found at page 4, lines 14-18. Claims 33, 34 and 36 have been amended to correct dependency. New Claims 40 and 43 have basis at page 14, last paragraph; new Claims 41, 44 and 46 have basis at page 4, lines 4-6 and original Claims 2, 14 and 18; new Claim 42 has basis at the paragraph bridging pages 4 and 5, page 9, second full paragraph and original Claim 13; new Claim 45 has basis at page 5, lines 14-26.

The present application was unintentionally abandoned due to non-response to the outstanding final Office Action mailed 9/25/98. Submitted concurrently herewith is a Petition to Revive Unintentionally Abandoned Application along with the appropriate fee.

A Notice of Appeal and request for oral hearing along with the appropriate fee is submitted concurrently herewith.

I. Section 112, 2nd paragraph rejection of Claims 32-27

The Examiner has rejected Claims 32-37 under Section 112, 2nd paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention. The Examiner states that Claim 32 is confusing. Applicants have amended the claim to recite "catalyzing reactions in the pathway of biosynthetic production of said desired compound" as suggested by the Examiner. Basis for this amendment occurs throughout the specification and in original Claim 13. Applicants believe that this claim amendment more clearly points out the subject matter that Applicant regards as the invention and therefore obviates the Examiner's concerns. Applicants respectfully request a withdrawal of this Section 112 second paragraph rejection of Claims 32-37 and as it might apply to any new claims.

I. II. The Claims are Novel

Claims 23, 27, 28 and 38 are rejected under Section 102(b) as being anticipated by Saier et al. Applicants respectfully traverse this rejection of claims.

Applicants submit that there can be no anticipation unless all of the same elements of the invention are found within the four corners of a single reference. Applicants submit that Saier et al., did not select for fast growing cells having a growth rate of at least about $0.4/hr^{-1}$ as required by the present invention. The Office Action states that this argument is not persuasive because the cells isolated by Saier et al., are fast growing. The Office action points to Table 1 which shows that the PTS-/glu+ mutants have a generation time of 2 hours.

Applicants believe that the Examiner may be confusing generation time with specific growth rate (μ) measured by h^{-1}. Applicants submit that these two terms are connected by the following formula: $\ln_2 / \mu$ (which represents natural logarithm to the base 2). See attached page 115 of Biochemical Engineering (Second Edition, *Academic Press, Inc.* 1973. New York and London) submitted herewith as Exhibit A.

Following this formula, a specific growth rate of 0.4 h^{-1} would have a generation time of 1.73 hours which is not disclosed in Saier et al. The specific growth rate of 0.8 h-1 would have a generation time of 0.87 hours which is not disclosed in Saier.

Thus, Claims 23, 27, 28, 38 and new Claim 42 require that the mutants selected are able to grow at least with a μ of $0.4h^{-1}$ which is equivalent to 1.73 hours. Saier disclosed mutants having at least a 2 hour generation time.

Therefore, Saier does not teach each and every element of the claimed invention. Given the strict standards for anticipation, it is readily apparent that there is no anticipation of the claimed invention in view of Saier et al. Applicants respectfully request a withdrawal of the Section 102 rejection of claims.

II. III. The Claims are Non-Obvious

The Office Action rejects claims 23-39 under Section 103 in view of the combined references Frost, Holms, Ingrahm et al., and Saier et at.

Applicants respectfully traverse this Section 103 rejection of claims.

M.P.E.P. Section 2141 states that when applying Section 103, the following tenets of patent law must be adhered to:

1) the claimed invention must be considered as a whole;
2) the references must be considered as a whole and suggest the desirability of making the combination;
3) the references must be viewed without the benefit of impermissible hindsight; and
4) reasonable expectation of success is the standard with which obviousness is determined.

As set forth in M.P.E.P. Section 2141.02, in determining the differences between the prior art and the claims, the question under Section 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. Stratoflex, Inc. v. Aeroquip Corp., 218 USPQ 871 (Fed. Cir. 1983). Furthermore, as stated in M.P.E.P. 706.02(j), in addition to providing motivation and a reasonable expectation of success, in order to meet the standard for obviousness, all claim limitations must be suggested by the prior art.

The Examiner's rejection was based on the subject matter of original Claims 1-4, directed to protein compositions and original Claim 8, directed to a recombinant vector comprising DNA encoding the protein composition of Claims 1-4.

Applicants submit that when viewed in its entirety, the presently claimed invention, i.e. host cells being phenotypically Pts-/glu+, requiring galactose permease activity to transport glucose; and having a specific growth rate on glucose as a sole carbon source of a least about $0.4h^{-1}$, and methods for making and using such host cells would not have been obvious in view of any of the references taken alone or in combination.

The skilled artisan following the teachings of Frost would be motivated to overexpress the transketolase gene (tkt) in a host cell as a means to increase carbon flow into the common aromatic pathway. Frost has no teachings or suggestions whatsoever regarding host cells modified to have a PTS-/glucose+ phenotype. Furthermore, the Office Action states at page 5, lines 10-16.

> The disclosure of Frost of amplification of carbon flow into the common aromatic pathway by increasing the amount of one of the substrates (E4P) for the first committed step of this pathway would suggest to the ordinary skilled artisan the

> amplification of the other necessary precursor (ie, PEP) of this enzymatic step as one this would assure that neither substrate for this enzyme would be in limiting supply.

Applicants fail to find a suggestion in Frost as to amplification of PEP for increasing flow into the aromatic pathway and respectfully invite the Examiner to point out the passages in Frost that suggest the "amplification of the other necessary precursor, i.e. PEP". Applicants believe the Examiner to be applying the impermissible use of hindsight reconstruction in making such an unsupported statement.

Assuming arguendo that such suggestions do exist in Frost, which Applicants don't concede, Applicants point out that the question is whether the claimed invention as a whole would have been obvious in view of Frost. The claimed invention is not directed merely to PEP amplification within a host cell but to host cells having a Pts-/glu+ phenotype, requiring galactose permease activity and having a specific growth rate on glucose as the sole carbon source of at least 0.4 h-1. There is no suggestion whatsoever in Frost of such a host cell. Furthermore, Applicants submit that there is no suggestion in Frost to combine the teachings of Frost with art related to phosphotransferase transport systems or with any of the cited art. One of skill in the art following the teachings of Frost alone or in combination with the cited references would not be expected to successfully arrive at the presently claimed invention.

Holms provides general discussion related to central metabolic pathways of E.coli and discusses the role of PEP in those pathways. The Examiner states at page 6, lines 1-4 that

> The disclosure of Holms that 66% of the cellular PEP is used by the competing PTS pathway would suggest to the ordinary skilled artisan that PEP availability to the common aromatic pathway could be substantially increased by preventing PEP use by the PTS pathway.

Applicants submit that Holms has no teachings or suggestions whatsoever regarding pathway engineering associated with increasing PEP availability in a host cell, and no teachings whatsoever regarding preventing PEP use by the PTS pathway. Applicants respectfully invite the Examiner to point out where in Holms it is suggested that the common aromatic pathway could be substantially increased by preventing PEP use by the PTS pathway. Furthermore, there are no teachings or suggestions regarding the

claimed invention as a whole, i.e. host cells having a Pts-/glu+ phenotype, requiring galactose permease activity and having a specific growth rate on glucose as the sole carbon source of at least 0.4 h-1, or methods for increasing PEP flow into a pathway or the production of desired compounds comprising the use of such a cell. Applicants fail to see any suggestions whatsoever within Holms to combine Holms with any other cited references.

Ingrahm relates to an alternative pathway for glucose transport, i.e. by uncoupling glucose transport into the cell from PEP utilization by relying on GLF and hexokinase, a pathway characteristic of Z.mobilis, see Ingrahm col. 3, lines 19-33 and col. 4, lines 13-23. Regarding Ingrahm, the Examiner states:

> Ingrahm et al., explicitly suggest this (preventing PEP use by the PTS pathway) as an approach to increasing the level of carbon flow into the common aromatic pathway.

Applicants fail to find any suggestion whatsoever in Ingrahm regarding increasing PEP availability to the cell by the use of a PTS-/glucose+ cell or of the claimed invention as a whole. In fact, one of skill in the art would be motivated to modify a host cell, such as E.coli, to comprise a glucose uptake pathway characteristic of Z. mobilis, i.e. transforming a host cell to comprise GLF and a hexokinase. Ingrahm reference a host cell mutant defective in the PTS system, see col. 5, lines 10-26, but fail to suggest or appreciate the relationship between PEP availability and the defective PTS system or the ability to utilize native E. coli genes to generate a glucose+ phenotype. One of skill in the art reading Ingrahm would be motivated to engineer a host cell to comprise GLF and hexokinase. Applicants find no suggestion in Ingrahm to combine Ingrahm with any other references.

The Office Action states at page 6, lines 7-11 that Saier et al., show that it is possible to produce cells which are deleted in the PTS system yet still retain high growth rates on glucose by using galactose permease as a means of glucose transport. Applicants submit that in spite of the Saier et al., discussion of host cells deficient in the PTS system, the growth rates of the Saier mutants do not suggest the claimed invention as a whole. Saier has no suggestion whatsoever of cells which have a specific growth rate on glucose as the sole carbon source of at least 0.4 h-1. Saier provides no motivation to one of skill in the art to produce such host cells or expectation of

successfully producing such cells. Furthermore, Saier et al., are absent any suggestions to combine the teachings of Saier et al., with any other cited art.

Applicants submit that combining reference teachings is improper unless the prior art suggests such a combination. As held in In re Bond, 910 F.2d 831, 15 U.S.P.Q.2d 1566 (Fed. Cir. 1990) the PTO erred in rejecting the claimed invention as an obvious combination of the teachings of two prior art references when the prior art provided no teaching suggestion or incentive supporting the combination. Applicants submit that it is improper to pick and choose among the individual elements of prior art references to recreate the claimed invention.

Applicants submit that this Section 103 rejection does not meet all the requirements of M.P.E.P. section 706.02(j) therefore, the Examiner has not succeeded in establishing a *prima facie* case of obviousness.

Assuming arguendo that there is a *prima facie* showing of obviousness, which Applicants do not concede, Applicants have presented evidence and arguments that rebut the showing.

In summary, Applicants submit that the presently claimed invention is non-obvious in view of the cited references because:

1. there is no motivation provided by any of the cited references alone or in combination to produce the claimed invention;

2. there is no suggestion of the claimed invention as a whole in any of the cited references. In fact, Applicants believe that Ingrahm et al., teach away from the presently claimed invention by suggesting the use of a pathway characteristic of Z.mobilis, i.e. by suggesting engineering a host cell to comprise GLF and hexokinase, rather than relying on native E. coli genes to generate a glucose+ phenotype. Applicants also believe that there is no teaching, suggestion or incentive supporting the combination of references; and

3. there is no expectation of successfully producing the claimed invention provided by the cited art.

Applicants believe that the presently claimed invention is non-obvious in view of the cited references and respectfully request a withdrawal of the Section 103 rejection of claims.

CONCLUSION

In view of the remarks provided herewith, Applicants submit that this application is in condition for allowance. Such action by the Examiner is earnestly solicited.

The Commissioner is hereby authorized to charge the fees necessitated by the filing of these documents, or to charge any additional fees under 37 C.F.R. 1.16 and 1.17 or to credit any overpayment, to Deposit Account No. 07-1048 (Docket No. GC266-2).

Respectfully submitted,

Date: October 12, 1999

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EXHIBIT A

BIOCHEMICAL ENGINEERING

Second Edition

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[redacted]

and product formation. In the case of unicellular growth, the growth rate of cells can be expressed in terms of the cell concentration, X, the concentrations of a growth-limiting substrate, S, (Note: there are cases where more than a single substrate can be growth limiting) and an inhibitor, I, i.e.,

$$\frac{dX}{dt} = f(X, S, I) \tag{4.36}$$

Generally, the inhibitor, I, in Eq. (4.36) may imply a predator, X_2 (see Section 4.5 later). In many instances the variables, X, S, and I are highly coupled. Hence, expressions for cell growth rate are usually highly nonlinear.

The specific growth rate, μ, is defined as

$$\mu \equiv \frac{1}{X}\frac{dX}{dt} \tag{4.37}$$

If the value of μ is constant, Eq. (4.37) represents the so-called exponential growth, where growth is proportional to the mass of cells present. Note that the specific growth rate is related to the mass-doubling time, t_d, by

$$t_d = 0.693/\mu = \frac{\ln 2}{\mu} \tag{4.38}$$

Growth other than the exponential type has been proposed. For example, linear growth (dX/dt=constant) occurs in some hydrocarbon fermentations where limitation is due to the rate of diffusion of substrate from oil droplets, provided their surface area is constant.[13,14] In filamentous organisms where growth occurs from the tip, but nutrients diffuse throughout the filamentous cell mass, the growth rate may be proportional to the surface area of mycelia or the 2/3rds power of the cell mass.

4.4.1. Expressions for μ

The following empirical equation has been commonly used to express the specific growth rate, μ,

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{4.39}$$[28]

where

μ_{max} = maximum specific growth rate
K_s = saturation constant
S = concentration of growth-limiting substrate

Equation (4.39) is analogous to Eq. (4.3) (Michaelis-Menten equation), but Eq. (4.39), the so-called Monod equation, has been derived empirically, while Eq. (4.3) is theoretical.